

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of

INZÉ et al. I Atty. Ref.: 5547-2; Confirmation No. 1234

Appl. No. 10/531,475 TC/A.U. 1638

Filed: April 15, 2005 Examiner: Collins

For: IDENTIFICATION OF NOVEL E2F TARGET GENES AND USE THEREOF

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Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

RULE 132 DECLARATION

I, Valerie Frankard, do hereby declare and say as follows:

1. I am an employee of CropDesign N.V., in Gent, Belgium, the Assignee of the above-identified application.
2. I have reviewed the above-identified application as well as the claims.
3. I am a Belgian citizen residing at Waterloo, Belgium.
4. From 1982-1987 I studied agronomical engineering and received a Master's Degree from the Université Libre de Bruxelles, in Brussels, Belgium. From 1987-1992, I studied Cell and Gene Biotechnology at the Vrije Universiteit Brussel, in Brussels, Belgium, where I earned my PhD degree. My thesis was in the field of plant nutritional quality improvement via biotechnology. From 1993-1999, I was a postdoctoral

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researcher and principal investigator (P1) at the Vrije Universiteit Brussel, in Brussels, Belgium.

5. From 1999 to 2009, I have held the position of Technology Management Coordinator of CropDesign N.V., in Gent, Belgium.

6. I am presently Senior Scientist, Yield Biology, and Research Manager of the Rice Yield Project at CropDesign.

7. I have reviewed the results submitted to the U.S. Patent Office in the above in remarks presented July 13, 2009. The results are presented again herein, with corrections, as work that I am familiar with and which has been performed to demonstrate that the claimed invention is supported by a disclosure which teaches one of ordinary skill in the art how to make and use the claimed invention, without requiring undue experimentation.

8. The previously-presented results were reported as including SEQ ID NO:1835. It has now been appreciated that the previously-presented results were conducted with a sequence referred to in the following alignment as CDSxx77, which comprises one mismatch to SEQ ID NO: 1835 within the coding sequence

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Needle SEQID1835-CDSxx77.txt

```
#####
# Program: needle
# Rundate: Fri 26 Mar 2010 14:36:47
# Commandline: needle
#   -auto
#   -asequence /srv/www/vhosts/embossgui/htdocs/output/832812/.asequence
#   -bsequence /srv/www/vhosts/embossgui/htdocs/output/832812/.bsequence
#   -brief
#   -outfile outfile
#   -aformat3 srspair
# Align_format: srspair
# Report_file: outfile
#####

=====
#
# Aligned_sequences: 2
# 1: CDSxx77
# 2: SEQID1835
# Matrix: EDNAFULL
# Gap_penalty: 10.0
# Extend_penalty: 0.5
#
# Length: 982
# Identity: 821/982 (83.6%)
# Similarity: 821/982 (83.6%)
# Gaps: 160/982 (16.3%)
# Score: 4101.0
#
#=====

CDSxx77      1 gtgcacaattgatgagcaatgttttataatgccaacttgtacaaaa    50
SEQID1835     0 -----          0
CDSxx77      51 aaggcaggcttaaacatggccctcgaaagcgatgaacactccaacttcttc 100
SEQID1835     1 -----atggccctcgaaagcgatgaacactccaacttcttc            35
CDSxx77      101 tttcaccagaatcgaaacgaaaagaagatgtatgaacgacgccgtttca 150
SEQID1835     36 tttcaccagaatcgaaacgaaaagaagatgtatgaacgacgccgtttca            85
CDSxx77      151 ttgagccgtggcttaaacgc当地acgc当地acgc当地acgc当地acgc 200
SEQID1835     86 ttgagccgtggcttaaacgc当地acgc当地acgc当地acgc当地acgc 135
CDSxx77      201 ccttcttc当地tcttccctc当地ccgc当地cgatctcg当地acccaaatccc当地 250
SEQID1835     136 ccttcttc当地tcttccctc当地ccgc当地cgatctcg当地acccaaatccc当地 185
CDSxx77      251 tcaagatcttacgaaagaagatgtatctcg当地tcttgc当地tctcatgctcg 300
SEQID1835     186 tcaagatcttacgaaagaagatgtatctcg当地tcttgc当地tctcatgctcg            235
CDSxx77      301 ctaaagatcaaccgtcg当地acgc当地gtatctcaacagtc当地caatcg当地 350
SEQID1835     236 ctaaagatcaaccgtcg当地acgc当地gtatctcaacagtc当地caatcg当地 285
CDSxx77      351 acgccc当地ccgc当地cagaatcaaagaaccttccgtacaagtgt当地acgtctgtga 400
SEQID1835     286 acgccc当地ccgc当地cagaatcaaagaaccttccgtacaagtgt当地acgtctgtga            335
```

Needle SEQID1835-CDSxx77.txt

CDSxx77	401	aaaagcgttccttcatcaggctttaggcggtcacaaagcaagtcacc	450
SEQID1835	336	aaaagcgttccttcatcaggctttaggcggtcacaaagcaagtcacc	385
CDSxx77	451	gaatcaaaccaccaaccgtaatctcaacaaccgcccattcaacagt	500
SEQID1835	386	gaatcaaaccaccaaccgtaatctcaacaaccgcccattcaacagt	435
CDSxx77	501	ccgaccatctccatcgtcgcccggagaaaaacatccgattgctgcctccgg	550
SEQID1835	436	ccgaccatctccatcgtcgcccggagaaaaacatccgattgctgcctccgg	485
CDSxx77	551	aaagatccacgagtgttcaatctgtcataaaagtgttccgacgggtcaag	600
SEQID1835	486	aaagatccacgagtgttcaatctgtcataaaagtgttccgacgggtcaag	535
CDSxx77	601	ctttaggcggtcacaaacgttgtcactacgaaggcaacctcgccggcga	650
SEQID1835	536	ctttaggcggtcacaaacgttgtcactacgaaggcaacctcgccggcga	585
CDSxx77	651	ggaggaggaggaagcaaatcaatcagtcacagtggaaagcgtgtcgagcac	700
SEQID1835	586	ggaggaggaggaagcaaatcaatcagtcacagtggaaagcgtgtcgagcac	635
CDSxx77	701	ggtatcggaaagaaaggagccaccgtggattcatcgatctaaccctaccgg	750
SEQID1835	636	ggtatcggaaagaaaggagccaccgtggattcatcgatctaaccctaccgg	685
CDSxx77	751	cgttacctgaactcagccttcatcacaatccaatcgtcgacgaagagatc	800
SEQID1835	686	cgttacctgaactcagccttcatcacaatccaatcgtcgacgaagagatc	735
CDSxx77	801	ttgagtccgttaccggtaaaaaaccgcctttgttaccgatcacgacca	850
SEQID1835	736	ttgagtccgttaccggtaaaaaaccgcctttgttaccgatcacgacca	785
CDSxx77	851	agtcatcaagaaaagaagatttcttaaaatctaatactcgacccag	900
SEQID1835	786	agtcatcaagaaaagaagatttatcttaaaatctaa-----	822
CDSxx77	901	ctttcttgtacaaagttggcattataagaaagcattgcttatcaatttgt	950
SEQID1835	822	-----	822
CDSxx77	951	tgcaacgaacaggtcacttcatcaataatatc	982
SEQID1835	822	-----	822

9. The peptide encoded by CDSxx77 is 99.6% similar to the peptide encoded by SEQ ID NO:1835 (i.e., SEQ ID NO:1836) as demonstrated by the following alignment:

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```
Needle SEQID1836-CDSxx77.txt
#####
# Program: needle
# Rundate: Wed Feb 13 16:08:53 2008
# Align_format: srspair
# Report_file: outfile
#####
=====
# Aligned_sequences: 2
# 1: SEQID1836
# 2: CDSxx77p
# Matrix: EBLOSUM62
# Gap_penalty: 11.0
# Extend_penalty: 1.0
#
# Length: 273
# Identity: 272/273 (99.6%)
# Similarity: 272/273 (99.6%)
# Gaps: 0/273 ( 0.0%)
# Score: 1427.0
#
#
=====

SEQID1836      1 MALEAMNTPTSSFTRIETKEDLMNDAVFIEPWLRKRKRSKRQRSHSPSSSS      50
CDSxx77p       1 MALEAMNTPTSSFTRIETKEDLMNDAVFIEPWLRKRKRSKRQRSHSPSSSS      50
SEQID1836      51 SSPPRSRPKSQNQDLTEEEYLALCLLMLAKDQPSQTRFHQQSQSLTPPPE 100
CDSxx77p       51 SSPPRSRPKSQNQDLTEEEYLALCLLMLAKDQPSQTRFHQQSQSLTPPPE 100
SEQID1836      101 SKNLPYKCNVCEKAFPSYQALGGHKASHRIKPPTVISTTADDSTAPTISI 150
CDSxx77p       101 SKNLPYKCNVCEKAFPSYQALGGHKASHRIKPPTVISTTADDSTAPTISI 150
SEQID1836      151 VAGEKHPIAASGKIHECSICHKVFPTGQALGGHKRCHYEGNLGGGGGGGS 200
CDSxx77p       151 VAGEKHPIAASGKIHECSICHKVFPTGQALGGHKRCHYEGNLGGGGGGGS 200
SEQID1836      201 KSISHSGSVSSTVSEERSHRGFIDLNLPALPELSLHHNPIVDEEILSPLT 250
CDSxx77p       201 KSISHSGSVSSTVSEERSHRGFIDLNLPALPELSLHHNPIVDEEILSPLT 250
SEQID1836      251 GKKPLLLTDHDQVIKKEDLSLKI    273
CDSxx77p       251 GKKPLLLTDHDQVIKKEDFSLKI    273
```

10. In view of this similarity, I believe that the previously presented results demonstrate that one of ordinary skill in the art could make and use the claimed invention without undue experimentation. The error in previously identifying the sequence of the previously presented results as SEQ ID NO:1835 occurred without deceptive intent.

11. Example A: CDSxx77 under the control of the constitutive promoter GOS2

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A DNA fragment encoding the 2XC2H2 protein represented in the application by CDSxx77p was isolated from an *Arabidopsis thaliana* seedling cDNA library (Invitrogen, Paisley, UK). PCR was performed using Hifi Taq DNA polymerase in standard conditions, using 200 ng of template in a 50 µl PCR mix. The primers used for the PCR amplification (and which include the AttB sites for Gateway recombination) were as follows:

Forward primer: ggggacaagggttacaaaaaagcaggctaaacaatggccctcgaaagcg

Reverse primer: ggggaccacttgtacaagaaaagctgggtcgagtattagattttaaagataaaatc

The amplified PCR fragment was purified also using standard methods. The first step of the Gateway procedure, the BP reaction, was then performed, during which the PCR fragment recombined *in vivo* with the pDONR201 plasmid to produce, according to the Gateway terminology, an “entry clone”. Plasmid pDONR201 was purchased from Invitrogen, as part of the Gateway® technology.

The entry clone comprising CDSxx77 was then used in an LR reaction with a destination vector used for *Oryza sativa* transformation. This vector contained as functional elements within the T-DNA borders: a plant selectable marker; a screenable marker expression cassette; and a Gateway cassette intended for LR *in vivo* recombination with the nucleic acid sequence of interest already cloned in the entry clone. A rice GOS2 promoter for constitutive specific expression was located upstream of this Gateway cassette. After the LR recombination step, the resulting expression vector (pGOS2::CDSxx77) was transformed into *Agrobacterium* strain LBA4044 according to methods well known in the art.

Rice transformation and phenotypic evaluation of the plants was as described in Example 12.

The results are shown in Table I below.

Table I: Results of phenotypic characterization of rice plants transformed with pGOS2::CDSxx77. Overall percentage of increase is given for biomass (above ground area and root area), total seed number and for flowers per panicle for T1 and T2 plants.

Parameter	T1 generation		T2 generation	
	% increase	p-value	% increase	p-value
Above ground area	10.5	0.002	9.5	0.000
Root area	7.4	0.01	4.5	0.0008
Plant height	5.5	0.000	2.8	0.000
Total number of seeds	9.7	0.031	11.9	0.001
Flowers per panicle	7.8	0.000	8.0	0.001

In addition, an increase was observed for seed fill rate (3 positive lines out of 4 in T2, overall increase of 30.1% with a p-value of 0.000), for harvest index (3 positive lines out of 4 in T2, overall increase of 35.0% with a p-value of 0.000) and for thousand kernel weight (2 positive lines out of 4 in T2, overall increase of 1.9% with a p-value of 0.005).

12. Example B: CDSxx77 under the control of the seed-specific promoter prolamin

Cloning of CDSxx77 was as described above. The entry clone was subsequently used in an LR reaction with a destination comprising the seed-specific prolamin promoter (mentioned in Example 12). Plant transformation and phenotypic analysis were as described above and as described in Example 12.

Table II: Results of phenotypic characterization of rice plants transformed with pPROLAMIN:: CDSxx77.

Parameter	T1 generation		T2 generation	
	% increase	p-value	% increase	p-value
Early vigour	25.7	0.001	10.4	0.021
Total seed weight	17.7	0.001	8.3	0.007
Total number of seeds	11.0	0.003	5.1	0.049
Seed fill rate	7.4	0.014	1.9	0.123
Harvest index	14.3	0.000	5.1	0.040
Number of filled seeds	19.5	0.000	7.2	0.018

In addition, an increase was observed for thousand kernel weight (2 positive lines out of 4 in T2, with respectively an increase of 3.1% with a p-value of 0.019, and an increase of 3.2% with a p-value of 0.013).

13. I believe that the above demonstrates that the claims are supported by a disclosure which teaches one of ordinary skill in the art how to make and use the claimed invention, without requiring undue experimentation.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false

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statements and the like so made are punishable by fine or imprisonment, or both, under
Section 1001 of Title 18 of the United States Code and that such willful false
statements may jeopardize the validity of the application or any patent issued thereon.

Signed this 21 day of May, 2010.

(Signature)

Valerie Frankard